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Comparison of the Toxicity Using Body Residues of DDE and Select PCB Congeners to the Midge, *Chironomus riparius,* in Partial-Life Cycle Tests

H. Hwang,[1] S. W. Fisher,[1,2] K. Kim,[2] P. F. Landrum[3]

[1] Environmental Science Graduate Program, The Ohio State University, 1735 Neil Avenue, Columbus, Ohio 43210, USA
[2] Department of Entomology, The Ohio State University, 1735 Neil Avenue, Columbus, Ohio 43210, USA
[3] Great Lakes Research Laboratory, NOAA, 2205 Commonwealth Boulevard, Ann Arbor, Michigan 48105, USA

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Abstract. Due to the long time course required to achieve steady state with highly lipophilic contaminants such as PCBs (polychlorinated biphenyls), data derived from short-term toxicity tests may lead to an erroneous interpretation of hazard. In addition, PCBs bioaccumulated over time can cause sublethal impairments in organisms at concentrations much lower than required for mortality. Here, the body residues of 1,1-dichloro-2,2-bis-*p*-chlorophenyl ethane (DDE) and select PCB congeners associated with a spectrum of chronic effects in the midge, *Chironomus riparius,* were evaluated. The route of exposure was ingestion of the PCB-contaminated alga, *Chlorella vulgarus,* and trout chow loaded with the selected test compound. Two separate exposures of midges were performed. In the first experiment, midges were exposed from the second instar to the pupal stage. In the second exposure, midges were exposed from the second instar to the adult stage. A variety of sublethal endpoints was monitored, including developmental time within a stadium, body weight, and fecundity for the female adult. The dose was assessed as the whole body residue concentration of the contaminant. Overall, the midge concentration increased with increasing exposure concentration in algae and trout chow. Body weight at the end of each stadium was the assessment parameter that was least significantly affected among the test endpoints monitored. In contrast, a significant increase in development time was the endpoint that was most frequently observed in response to contaminant exposure. Reduction in fecundity was found only for DDE-exposed midges. These data, in which chronic endpoints are related to body residues, suggest that body residues will be useful in defining sublethal hazards of DDE and some PCB congeners.

Neutral lipophilic compounds (NLCs) such as polychlorinated biphenyls (PCBs) and other organochlorines continue to adversely affect the integrity of the ecosystems (Hooper *et al.* 1990; Tillitt *et al.* 1992), even though their direct inputs to ecosystems have dramatically decreased (Baumann and Whittle 1988; Gilbertson 1983). Moreover, there is evidence that organisms that dwell in or are in contact with sediment can be adversely affected, even in the regions where the USEPA Water Quality Criteria (WQC) for NLCs are not exceeded (Chapman 1989). In fresh lentic water bodies, sediments are known as a primary sink for NLCs. For instance, 97% of released PCBs are associated with sediments or other particles, and PCB concentrations on the particles are higher than in overlying water by three orders of magnitude (DiPinto *et al.* 1993). Sediment bound NLCs can slowly desorb and be transferred to the biota, giving a variety of adverse effects to individual organisms (de Solla *et al.* 2002; Weseloh *et al.* 2002) and whole aquatic ecosystems (Hoagland *et al.* 1993; Sugiura 1992). However, total concentrations of contaminants in the sediment have not been strongly correlated with the consequent effects or with the extent of bioaccumulation because variation in bioavailability can arise from characteristics of sediment, the nature of the interaction between biota and sediment, and the interaction of the contaminant with a particular sediment (Burton 1991).

A variety of means exist for assessing the adverse effects of contaminants on aquatic organisms including: laboratory bioassays, *in situ* bioassays, and benthic community analyses (Burton 1991; Harkey *et al.* 1994a; Canfield *et al.* 1996). The need for a wide range of approaches to assess sediment-associated contaminants exists because of the differential bioavailability of the contaminants in sediments. The differences in bioavailability, in turn, may stem from differences in behaviors and the nature of the interaction between sediment and organisms (Hamelink *et al.* 1994). For instance, three benthic organisms, *Chironomus riparius, Lumbriculus variegatus,* and *Diporeia* spp., exposed to sediments spiked with pyrene, benzo[*a*]pyrene, and *trans*-chlordane, exhibited significantly different levels of bioaccumulation, with *C. riparius* showing the lowest bioaccumulation and *Diporeia* spp. the highest (Harkey *et al.* 1994b). This was attributed to differences in feeding selectivity, lipid content, and physiological/metabolic variation

Correspondence to: Dr. Susan W. Fisher, Department of Entomology, The Ohio State University, 1735 Neil Avenue, No. 103, Columbus, OH 43210, USA; *email:* fisher.14@osu.edu

between organisms. Similar results were obtained when a series of invertebrates with varying feeding habits was exposed to sediments contaminated with chlordane. In this case, surface-feeders or surface-dwellers exhibited higher bioaccumulation than suspension feeders by a factor of three (Wilcock *et al.* 1993). Further, there were significant differences in bioavailability among classes of compounds, with *trans*-chlordane exhibiting much greater bioavailability than benzo[*a*]pyrene (Harkey *et al.* 1994b). Similar results have been observed for a range of sediments (Landrum *et al.* 1997). These examples clearly demonstrate that differences in the ways organisms interact with the environment and/or characteristics of the environment and the contaminant can cause significant changes in exposure and subsequent bioaccumulation. Basing hazard assessments on toxicity data obtained from one or a few species may result in erroneous assessments of hazard. Even techniques designed to attenuate the interference of key variables, *e.g.*, carbon normalization or adjusting for organism lipid, are not always successful (Ingersoll *et al.* 1996; Landrum *et al.* 1997). Thus, the relationship between contaminant concentration in sediment and toxicity remains difficult to predict despite the pressing need to establish sediment quality criteria guidelines for organic contaminants (McDonald *et al.* 2000).

Using body residues instead of the environmental concentrations may circumvent the difficulties associated with differential bioavailability. The environmental concentration has typically been used as a surrogate for exposure in aquatic systems. When toxicity testing was conducted using water as the delivery route, it was well recognized that the concentration in the water was proportional to that in the organisms, which in turn was proportional to the dose at the receptor. However, when the contaminant source is sediment, variable bioavailability in sediment tends to confound the straightforward relationship determined between bioconcentration and contaminant lipophilicity established from water-borne exposures. Thus, using body residue as the dose metric, which is only one step removed from the receptor dose, will eliminate the need to account for bioavailability. Use of body residues also accounts for exposure that may occur from more than one route of exposure. Body residues are still surrogates for the concentration at the target site, but should reflect only toxic potential and vary only by genetic differences of the organisms in susceptibility to the contaminants, not by bioaccumulation potential (Fisher *et al.* 1999). In fact, body residues have been successfully used in assessing environmental hazard of the contaminants associated with sediments (Landrum *et al.* 1994; Lotufo 1998; van Wezel and Jonker 1998; Lotufo *et al.* 2001). However, the availability of body residue data, especially for assessing nonlethal effects, is still too limited to be used in ecological risk assessment.

This work compares sublethal impairments of growth, developmental time, and fecundity of DDE and select PCB congeners in the midge, *Chironomus riparius,* using body residue as the dose metric. A toxicity spectrum is established for the sublethal endpoints that will allow assessment over a range of body residues and permit comparison of the sublethal responses to mortality data collected earlier (Hwang 2000).

Materials and Methods

Chemicals

The chemicals studied were DDE (specific activity, 12.7 mCi/mmol), 4,4′-dichlorobiphenyl (DCBP; specific activity, 13.8 mCi/mmol), 2,2′,4,4′-tetrachlorobiphenyl (TCBP; specific activity, 18.5 mCi/mmol), and 3,3′,4,4′,5-pentachlorobiphenyl (PCBP; specific activity, 21.2 mCi/mmol). These chemicals were chosen because of their availability in radiolabeled form and environmental significance. Stock solutions for exposures were made by combining both ^{14}C-labeled and nonradiolabeled compounds. The specific activities of the stocks were then recalculated to reflect isotopic dilution. The radiopurity of all compounds was determined using thin layer chromatography (TLC) by applying a small amount of stock solution of each contaminant to a silica gel GF 254 plastic-backed TLC plate. The TLC plates were developed in a solvent system consisting of hexane:benzene 80:20. Parent compounds were identified by co-migration of cold standards of each contaminant. The silica gel corresponding to parent compound was cut from the plate and placed into a scintillation vial with 5 ml of ^{14}C-cocktail. Thereafter, the remaining silica gel below each parent compound spot was divided into 1-cm sections and placed in separate scintillation vials with 5 ml of cocktail. The radioactivity in each section of silica gel was quantified using a liquid scintillation counter (LSC; 6000 LSC; Beckman, Fullerton, CA) with automatic quench correction. Percent purity was calculated as the ratio of radioactivity due to parent compound to total radioactivity from all silica gel sections. The PCBs were >98.0% pure and DDE was 96.6% pure. All ^{14}C-compounds were purchased from Sigma Chemical Co. (St. Louis, MO) and all ^{12}C-compounds were purchased from Chem Service (West Chester, PA).

Organisms

The midge, *Chironomus riparius,* was used as the test organism. *C. riparius* was reared in the Environmental Toxicology Laboratory at The Ohio State University according to the method of Estenik and Collins (1978). The midge larvae were initially collected in 1974 from the Jackson Pike Water Treatment Plant in Columbus, OH and have been maintained in culture with periodic out breeding to wild midges. Midges were chosen as test organisms because they are sediment-dwelling, easy to culture, ecologically important, and have a broad geographical distribution (USEPA 2000).

Media

Hard standard reference water (HSRW)—pH 8.3–8.5; alkalinity, 120 mg/L as $CaCO_3$; and hardness, 160 mg/L as $CaCO_3$—was used in all experiments (USEPA 1975). Tap water was processed through a series of filters and resins to produce deionized water. The necessary salts were predissolved in smaller vessels and then added to the deionized water to produce HSRW. The water was mixed overnight and the required water quality parameters (pH, hardness and alkalinity) were confirmed according to laboratory standard operating procedures.

In general, contaminant concentrations in algae are expressed as mg of contaminant per kg of algal mass as is typical for representation of contaminant concentrations in environmental media. In contrast, we have generally expressed tissue concentrations of contaminants in midges on a molar basis, which is consistent with the emerging literature on critical body residues. Environmental concentrations can be converted to molar concentrations by dividing by the molecular

weight (g/mol) of each contaminant, which are as follows: DDE, 304; MCBP, 185; DCBP, 216; TCBP, 278; PCBP, 309.

Feeding Midges

The green alga, *Chlorella vulgaris,* and commercial trout chow were used to deliver the contaminants to the midges. *C. vulgaris* is a round alga that does not have cilia or flagella for locomotion and was chosen because it settles quickly and is readily ingested by midges.

A pure stock of *C. vulgaris,* purchased from Carolina Biological Supply (Burlington, NC), was cultured in Bold's Basal medium (James 1978). Cultures were kept in an environmental chamber at 20°C under fluorescent lights with a photocycle of 14:10 light:dark. Air was bubbled mildly through glass tubes to suspend the algae cells in the water column.

Algae were spiked with the toxicant dissolved in acetone at a series of predetermined contaminant concentrations. The final contaminant concentration on algae was measured using LSC. The algal cell concentrations used in all tests was 2 million cells/mL. The number of algae was quantified on a Coulter Counter (Coulter Electronics Inc., Hialeah, FL). To ensure accurate spiking of algae, an aliquot of algae was filtered through microfilter paper (0.5 μm, 25 mm; MSI, Westdoro, MA) and dried overnight in an oven at 50°C to obtain an accurate dry weight. Dry algal weight was estimated from the difference in filter paper weight before and after filtering the algae. Then the amount of contaminant needed to obtain a series of predetermined concentrations was calculated. Thereafter, a mixture of radiolabeled and non-radiolabeled contaminant of each type was dissolved in acetone and put into Ehrlenmeyer flasks at the predetermined concentration series. To each flask, a known mass of algae in a minimal amount of algal medium were added. The algae were agitated for 12 h on a rotary shaker. Trout chow, spiked at the concentrations corresponding to those in algae and prepared in the same way, was also added to the test beakers to provide burrowing material and extra food. Trout chow was sieved using a No. 35 mesh (500 μm) and then defatted by soaking in ether for 30 min with gentle swirling before spiking with contaminant. Ether was removed with a rotary evaporator. The final concentration of contaminant from 10 mg of algae or trout chow was quantified using LSC. Fresh contaminated media were prepared every 3–5 days.

Partial-Life Cycle Tests

The experimental design followed that of Hwang et al. (2001). Five treatment groups and a control were used for all partial-life cycle tests. The contaminant concentrations in algae and trout chow for each chemical were based on a previously determined 10-day toxicity test (Hwang 2000). The chronic effects of the contaminants were evaluated in two different assays: The first exposed midges from the second instar to the pupal stage and the second exposed midges from the second instar to the adult stage. In both cases, exposure began with mixed sex second instar midges, which were the youngest stage that can endure handling stress. The second instar to pupae test ended when the midges reached pupal stage and the second instar to adult test ended when the midges eclosed to adults. Tests were initiated by placing 30 second-instar larvae individually into 30 50-mL beakers in static renewal tests. The number of organisms necessary for the test was calculated according to Dean and Voss (1995) to assure the statistical power of the data. Organisms were examined daily for mortality, and pupation in the second instar to pupae tests or emergence in the second instar to adult tests. In the second instar to adult tests, the number of ova in ovarioles was counted for each female adult. In both pupal and adult midges, the weight at the end of each stadium and developmental time for each stadium were determined. Midges that died during exposure were weighed and the body residue was measured using liquid scintillation counting (LSC). All midges that turned to the desired stadium were analyzed for body residues of each test chemical. The test endpoints for the second instar to pupae test were developmental time and larval weight. Endpoints for the second instar to adult test were developmental time, fecundity as defined by number of ova, and weight. Tests were performed at 20°C with a photoperiod of 14:10 h light:dark.

Contaminant Analysis

For midges, body residues were determined by LSC after weighing. Whole midges were individually transferred to scintillation vials, and 0.5 mL of tissue solubilizer (Packard Instrument Company, Inc., Meriden, CT) was added. After digestion, 5 mL of scintillation cocktail (1,4-dioxane [1000 mL], naphthalene [100 g], and 2,5-diphenoloxazole [PPO; 5 g]) was added and vials were allowed to stand overnight to reduce the chemiluminescence. The samples were then counted by LSC for 5 min per vial.

Data Analysis

For the partial-life cycle test, data from each exposure concentration were pooled and subjected to the general linear model (GLM) analysis followed by Duncan's multiple range test on SAS (SAS Institute Inc. 1992) to determine the lowest observed effective residue (LOER) in each endpoint. The null hypothesis of no difference was rejected at a p value of 0.05.

Results

Exposure Concentration and Body Residues

The effective contaminant concentrations (lowest contaminant concentration on media to produce an effect significantly different from controls), based on exposure concentration in algae and trout chow used in the tests, varied substantially among different contaminants. DCBP required the highest exposure concentration (ranging from 81.9 to 706.7 mg/kg in the second instar to pupae test and from 513 to 4693 mg/kg in the second instar to adult test), while TCBP required the lowest concentration (ranging from 0.88 to 8.76 mg/kg in the second instar to pupae test and 0.55 to 8.57 mg/kg in the second instar to adult test) (Tables 1–4). For DDE and TCBP, the contaminant concentrations in algae needed to produce a significant effect in the second instar to pupae test were similar to those in the second instar to adult test, while for DCBP and PCBP, effective contaminant concentrations in algae in the second instar to adult test were substantially higher than those in the second instar to pupae test (Tables 1–4). The variable exposure concentrations among contaminants needed to produce a significant biological response provide further impetus to investigate using body residues as a dose metric to potentially minimize such variability.

Body residues of contaminants increased with increasing algal contaminant concentration and varied significantly among contaminants and between developmental stages (Tables 1 and 2). The contribution of radioactivity associated with unabsorbed algae and trout chow in the gut was estimated to be less

Table 1. The average contaminant concentration in algae (mg/kg ± SD) used as food source and contaminant delivery medium for the second instar to pupa and second instar to adult tests

Test type	Contaminant concentration No.[a]	DDE	DCBP	TCBP	PCBP
2nd instar to pupa	1	1.61 ± 0.279	81.9 ± 13.3	0.88 ± 0.07	0.58 ± 0.22
	2	2.53 ± 0.246	161.8 ± 8.8	1.71 ± 0.17	1.62 ± 0.34
	3	3.52 ± 0.315	339.8 ± 27.5	4.16 ± 0.88	4.87 ± 0.95
	4	5.22 ± 0.447	484.0 ± 35.0	6.44 ± 0.57	6.79 ± 1.52
	5	10.23 ± 1.281	706.7 ± 41.4	8.76 ± 0.75	8.96 ± 2.20
2nd instar to adult	1	1.59 ± 0.41	513 ± 173	0.55 ± 0.11	1.73 ± 0.47
	2	2.52 ± 0.66	978 ± 325	1.74 ± 0.21	3.99 ± 0.56
	3	3.62 ± 1.98	1420 ± 450	4.64 ± 0.28	10.46 ± 1.25
	4	5.90 ± 1.30	2540 ± 805	6.67 ± 0.57	16.03 ± 2.07
	5	11.09 ± 3.00	4693 ± 1664	8.57 ± 1.25	21.74 ± 2.33

[a] Exposure concentrations of each contaminant on algae and trout chow are identified to the right of this column in Table 1. In subsequent tables, only the number in this column is used to identify exposure concentrations.

than 5% using the method of Bruner *et al.* (1994) and was, thus, not corrected in the total body residues.

Differences in the contaminant concentrations in the algae were not completely reflected in the body residues in midges. In the second instar to adult test, the DCBP algal exposure concentrations needed to cause significant effects were approximately 100-fold higher than the TCBP algal exposure concentrations. However, the body residues in the midges for the two contaminants differed by less than a factor of 10 (Tables 1 and 2). The apparent discrepancy is probably due to a much higher elimination rate for DBCP than TCBP in invertebrates (Fisher *et al.* 1999). Further, the body residues in the second instar to adult test were always higher than those in the second instar to pupae test, even when the contaminant concentrations in the algae were similar (Table 2). This may result from the longer exposure to the contaminants in the second instar to adult test.

Body Mass

Except for the pupae in the DDE tests, the sex of the pupae was determined and the body mass is, thus, presented by sex (Table 3). Data are presented as mean ± SD. When the body masses of midges were compared by sex, female midges were always heavier than male midges. For instance, female pupae in DCBP test weighed from 3.61 ± 0.44 to 4.20 ± 0.64 mg across the exposure doses tested, while male pupae varied only from 2.74 ± 0.43 to 3.31 ± 0.62 mg. Also, the weights of all female adults ranged from 2.61 ± 0.4 to 3.1 mg, while the weights of all males ranged from 1.52 ± 0.4 to 2.10 ± 0.8 mg.

Pupae were consistently heavier than adult midges (Table 3). For instance, pupae in the TCBP tests weighed from 4.65 ± 0.91 to 5.95 ± 0.91 mg and from 3.23 ± 0.76 to 4.54 ± 0.82 mg for female and male midges, respectively, while adult midges weighed from 2.04 ± 0.26 to 2.51 ± 0.39 mg and from 1.32 ± 0.17 to 1.71 ± 0.28 mg for female and male adults, respectively. The midges in the other tests showed the same trend.

Significant difference in body weight associated with changing body residues were found in pupal and male adult midges exposed to TCBP and male adults exposed to DCBP and PCBP (Table 3). Where significant differences in weights were found, the weights of the exposed midges were significantly greater than those of the controls. These differences were considered artifactual, created by very high mortality at high exposure concentrations in the later stages of the experiment in midges exposed to DCBP.

Developmental Time

When the developmental times were compared between sexes, the developmental time for female midges was consistently longer than for males for both pupae and adults (Table 4). Data are reported as mean ± SD. For instance, in the second instar to pupae test, female midges in the control pupated in 16.6 ± 1.3 to 18.3 ± 1.4 days across tests, while male midges in the control pupated in 14.0 ± 1.0 to 16.0 ± 1.6 days. In the second instar to adult test, female midges in the controls eclosed in 19.1 ± 2.2 to 19.7 ± 2.7 days, while their male counterparts eclosed in 16.4 ± 2.6 to 19.3 ± 4.4 days.

Increases in developmental time were the most consistently observed endpoint to be impacted by contaminants, among the chronic endpoints used to assess the effects of the contaminants (Table 4). Statistically significant changes in developmental time of pupal midges were found at body residues of 0.44 ± 0.08, 0.24 ± 0.04, 0.20 ± 0.07, 0.019 ± 0.06, 0.015 ± 0.024, and 0.0098 ± 0.003 mmol/kg for DCBP in females, TCBP in females, TCBP in males, PCBP in females, PCBP in males, and DDE in both sexes, respectively. In the second instar to adult tests, statistically significant changes in adult developmental time were found at body residues of 3.04, 1.92 ± 0.73, 0.063 ± 0.019, 0.054 ± 0.014, 0.051 ± 0.008, 0.023 ± 0.004, and 0.003 ± 0.0004 mmol/kg for DCBP in females, DCBP in males, PCBP in males, TCBP in females, PCBP in females, DDE in males, and DDE in females, respectively.

Fecundity

Fecundity was assessed only in adult midges (Table 5). The number of ova in control midges ranged from 297.2 ± 50.7 in

Table 2. Average body residues (mmol/kg ± SD) of test chemicals in midges

Test type	Contaminant concentration on algae[a]	DDE		DCBP		TCBP		PCBP	
		Female	Male	Female	Male	Female	Male	Female	Male
2nd instar to pupa	1	0.0010 ± 0.0004		0.12 ± 0.03	0.06 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.0010 ± 0.0004	0.0009 ± 0.0003
	2	0.0017 ± 0.0007		0.27 ± 0.08	0.14 ± 0.03	0.06 ± 0.01	0.04 ± 0.02	0.0027 ± 0.0007	0.0021 ± 0.0005
	3	0.0027 ± 0.0013		0.44 ± 0.08	0.23 ± 0.07	0.14 ± 0.03	0.10 ± 0.03	0.0078 ± 0.0012	0.0060 ± 0.0023
	4	0.0044 ± 0.0021		0.90 ± 0.20	0.33 ± 0.03	0.24 ± 0.04	0.15 ± 0.06	0.0135 ± 0.0020	0.0105 ± 0.0034
	5	0.0098 ± 0.0033		1.54 ± 0.34	0.75 ± 0.06	0.27 ± 0.03	0.20 ± 0.07	0.0191 ± 0.0055	0.0155 ± 0.0024
2nd instar to adult	1	0.003 ± 0.0004	0.003 ± 0.0013	0.41 ± 0.11	0.34 ± 0.05	0.054 ± 0.014	0.036 ± 0.026	0.003 ± 0.0006	0.003 ± 0.001
	2	0.006 ± 0.0042	0.006 ± 0.0027	1.02 ± 0.13	0.84 ± 0.20	0.127 ± 0.035	0.105 ± 0.032	0.007 ± 0.001	0.008 ± 0.001
	3	0.009 ± 0.0015	0.007 ± 0.0017	1.50 ± 0.31	1.17 ± 0.21	0.271 ± 0.086	0.236 ± 0.078	0.021 ± 0.006	0.023 ± 0.004
	4	0.016 ± 0.0018	0.012 ± 0.0036	3.04	1.92 ± 0.73	0.455 ± 0.064	0.388 ± 0.147	0.033 ± 0.007	0.035 ± 0.007
	5	0.034 ± 0.0062	0.023 ± 0.0022	N/A[b]	N/A	0.550 ± 0.095	0.431 ± 0.690	0.051 ± 0.008	0.063 ± 0.019

Midges were not separated by sex in the first DDE test.
[a] Contaminant concentrations on algae for each number are identified in Table 1.
[b] No midges successfully eclosed to adult.

TCBP exposures to 374 ± 57.7 in PCBP-treated midges. A statistical reduction in fecundity was found only for DDE-exposed animals at a body residue of 0.003 ± 0.0004 mmol/kg.

Spectrum of the Effects

The spectrum of chronic effects of DDE and select PCB congeners on pupal and adult midges is summarized in Figure 1. The significant changes in the pupal stage were found only in developmental time and ranged between 0.0098 and 0.44 mmol/kg. The most toxic contaminant to pupal midges, in terms of increase in developmental time, was DDE, with the lowest observed effect body residue (LOER) of 0.0098 (0.008–0.012) mmol/kg. In contrast, the least toxic contaminant was DCBP with an LOER of 0.44 (0.36–0.51) mmol/kg in female pupae. LOERs of test chemicals to adult midges were more widely spread, ranging from 0.0025 (0.0020–0.0031) to 3.04 mmol/kg. Of all the chemicals tested, DDE was the most biologically active contaminant, showing an LOER of 0.0025 mmol/kg for female adult developmental time and fecundity and an LOER of 0.023 (0.010–0.035) mmol/kg LOER for male adult developmental time. DCBP was the least biologically active contaminant tested, with an LOER of 1.92 (1.01–2.83) mmol/kg for male adult developmental time and an LOER of 3.04 mmol/kg for female developmental time and fecundity change.

In addition to other endpoints, significant mortality was also observed in the DCBP tests. No mortality was observed in tests with other contaminants. The 35-day LR_{50} (95% confidence interval) for DCBP was estimated to be 0.68 (0.43–1.01) mmol/kg, which is lower than the LOERs for fecundity and developmental time increases for DCBP by factors of approximately 4.5 and 2.8, respectively, for females and males (Figure 1).

Discussion

The route of exposure for this study was via the food in the forms of contaminated algae and trout chow. While it is likely that some contaminant sorbed to algal particles, desorbed from the particles, and entered the aqueous phase, exposure of the midges through contaminated algae is still considered the dominant route of contaminant exposure for midges. This route of exposure was dominant because of the limited water solubility of several of the compounds of interest. However, when body residue is used as the dose metric, the route of exposure becomes relatively unimportant, since it uses the midge to integrate exposure from all routes. It is, thus, unimportant to track exposure concentrations in diverse media or account for potential differences in bioavailability in different media. Measurement of the final concentration in the midge as the estimate of exposure allows one to bypass complex and expensive experimental measurements.

The use of contaminated algae as the primary exposure vehicle necessitated performing experiments of longer duration than standard toxicity tests. If the exposure duration were short (*e.g.*, 1–2 days), the rate of contaminant accumulation might be limited by necessity for contaminant to be desorbed from

Table 3. Average body mass of midges (mg ± SD); Numbers with different superscripts within a column are statistically different

Test type	Contaminant concentration on algae	DDE		DCBP		TCBP		PCBP	
		Female	Male	Female	Male	Female	Male	Female	Male
2nd instar to pupa	0			4.20 ± 0.64^{a}	3.16 ± 0.54^{a}	4.89 ± 0.70^{a}	3.56 ± 0.36^{ab}	4.53 ± 0.56^{a}	3.63 ± 0.32^{a}
	1	4.35 ± 1.74^{a}		4.20 ± 0.64^{a}	3.31 ± 0.62^{a}	4.76 ± 0.47^{a}	3.23 ± 0.76^{a}	4.44 ± 0.61^{a}	3.59 ± 0.44^{a}
	2	4.58 ± 1.27^{a}		4.00 ± 0.77^{a}	3.30 ± 0.46^{a}	4.89 ± 1.11^{a}	3.85 ± 0.70^{b}	4.69 ± 0.66^{a}	3.49 ± 0.95^{a}
	3	4.38 ± 1.18^{a}		3.94 ± 0.71^{a}	2.95 ± 0.28^{a}	4.65 ± 0.91^{a}	3.62 ± 0.55^{ab}	4.72 ± 0.63^{a}	3.58 ± 1.11^{a}
	4	4.35 ± 1.09^{a}		3.61 ± 0.44^{a}	2.74 ± 0.43^{a}	5.00 ± 0.62^{a}	4.04 ± 0.61^{ab}	4.77 ± 0.69^{a}	3.61 ± 0.71^{a}
	5	4.12 ± 0.64^{a}		3.74 ± 0.65^{a}	2.96 ± 0.28^{a}	5.95 ± 0.91^{b}	4.54 ± 0.82^{c}	4.20 ± 0.50^{a}	3.29 ± 0.46^{a}
2nd instar to adult	0	2.30 ± 0.02^{a}	1.47 ± 0.59^{a}	2.61 ± 0.4^{a}	1.52 ± 0.4^{a}	2.10 ± 0.36^{a}	1.32 ± 0.17^{a}	2.61 ± 0.41^{a}	1.34 ± 0.33^{a}
	1	2.39 ± 0.24^{a}	1.27 ± 0.20^{a}	2.62 ± 0.3^{a}	1.52 ± 0.2^{a}	2.04 ± 0.26^{a}	1.38 ± 0.38^{ab}	2.90 ± 0.47^{a}	1.69 ± 0.25^{c}
	2	2.38 ± 0.32^{a}	1.25 ± 0.34^{a}	2.93 ± 0.3^{a}	1.61 ± 0.3^{ab}	2.47 ± 0.44^{a}	1.46 ± 0.30^{abc}	2.53 ± 0.45^{a}	1.44 ± 0.14^{b}
	3	2.02 ± 0.38^{a}	1.47 ± 0.44^{a}	2.84 ± 0.5^{a}	1.73 ± 0.3^{ab}	2.35 ± 0.32^{a}	1.67 ± 0.22^{bc}	2.46 ± 0.30^{a}	1.44 ± 0.21^{ab}
	4	2.10 ± 0.22^{a}	1.42 ± 0.13^{a}	3.1*	2.10 ± 0.8^{b}	2.51 ± 0.39^{a}	1.38 ± 0.30^{ab}	2.43 ± 0.58^{a}	1.62 ± 0.30^{bc}
	5	2.13 ± 0.23^{a}	1.46 ± 0.42^{a}	N/A	N/A	2.49 ± 0.19^{a}	1.71 ± 0.28^{c}	2.45 ± 0.28^{a}	1.56 ± 0.29^{abc}

N/A: No midges successfully eclosed to adults. Contaminant concentrations on algae corresponding to each number are identified in Table 1. Midges were not separated by sex in the first DDE test.
* Only one female midge successfully eclosed to an adult.

Table 4. Developmental time of midges(average number of days ± SD); Numbers with different superscripts within a column are statistically different

Test type	Contaminant concentration on algae	DDE		DCBP		TCBP		PCBP	
		Female	Male	Female	Male	Female	Male	Female	Male
2nd instar to pupa	0	15.40 ± 4.16^{a}		16.6 ± 1.3^{a}	14.0 ± 1.0^{a}	18.2 ± 1.1^{a}	16.0 ± 1.6^{a}	18.3 ± 1.4^{a}	15.4 ± 1.2^{a}
	1	16.31 ± 4.41^{ab}		17.5 ± 1.9^{ab}	15.3 ± 2.1^{a}	18.7 ± 2.0^{a}	14.6 ± 1.7^{b}	18.3 ± 2.1^{a}	15.9 ± 1.7^{a}
	2	16.78 ± 4.96^{ab}		18.0 ± 2.6^{ab}	14.2 ± 1.3^{a}	20.7 ± 1.7^{b}	19.0 ± 2.3^{c}	19.3 ± 1.7^{a}	15.3 ± 1.7^{a}
	3	18.35 ± 4.29^{ab}		18.7 ± 1.0^{b}	17.0 ± 3.0^{a}	19.8 ± 2.3^{ab}	16.1 ± 1.2^{a}	18.3 ± 0.6^{a}	15.2 ± 2.0^{a}
	4	18.65 ± 5.22^{ab}		18.2 ± 2.2^{ab}	16.3 ± 3.2^{a}	22.4 ± 1.9^{c}	17.3 ± 1.8^{a}	18.9 ± 2.0^{a}	16.6 ± 2.9^{a}
	5	19.22 ± 4.54^{b}		21.1 ± 1.8^{c}	15.8 ± 0.9^{a}	23.8 ± 2.0^{c}	19.5 ± 2.2^{c}	22.1 ± 1.6^{b}	18.4 ± 2.5^{b}
2nd instar to adult	0	19.5 ± 2.5^{a}	16.4 ± 2.6^{a}	19.7 ± 2.5^{a}	19.3 ± 4.4^{a}	19.4 ± 2.7^{a}	17.1 ± 1.2^{a}	19.1 ± 2.2^{a}	16.8 ± 1.4^{a}
	1	27.8 ± 1.2^{b}	19.3 ± 5.9^{a}	25.9 ± 4.1^{bc}	17.5 ± 3.1^{a}	22.1 ± 2.5^{b}	16.1 ± 6.4^{a}	20.6 ± 2.0^{a}	18.3 ± 1.7^{a}
	2	25.2 ± 5.26^{b}	17.3 ± 6.6^{a}	24.1 ± 2.8^{ab}	19.3 ± 3.3^{a}	24.2 ± 4.2^{b}	19.4 ± 2.8^{a}	19.5 ± 2.4^{a}	17.2 ± 1.4^{a}
	3	25.19 ± 6.0^{b}	18.5 ± 8.7^{a}	23.2 ± 3.0^{ab}	19.4 ± 4.9^{a}	24.2 ± 3.5^{b}	20.0 ± 3.5^{a}	20.8 ± 1.3^{a}	17.8 ± 1.8^{a}
	4	27.1 ± 4.2^{b}	19.5 ± 6.7^{a}	29.0^{c}	26.6 ± 4.6^{b}	27.2 ± 3.3^{c}	21.0 ± 6.0^{a}	19.9 ± 1.8^{a}	17.8 ± 1.6^{a}
	5	28.0 ± 6.6^{b}	28.3 ± 6.8^{b}	N/A	N/A	26.9 ± 3.9^{c}	19.0 ± 5.5^{a}	22.9 ± 2.6^{b}	19.9 ± 1.9^{b}

N/A: No midges successfully eclosed to adults. Contaminant concentrations on algae corresponding to each number are identified in Table 1. Midges were not separated by sex in the first DDE test.

ingested algal particles, absorbed across the gut epithelium, and transported through the blood to active sites in the midge. In contrast, the use of aqueous exposures to generate an biologically effective tissue concentration would be constrained by the low water solubility of the contaminants under study. Both obstacles could be overcome by using the food-borne exposure in relatively long-term experiments (10–35 days) and, thus, was selected for use here.

Although, mortality is typically measured in toxicity tests, death is a crude estimate of effect. Clearly, profound ecological consequences of contaminant exposure may be realized long before affected organisms begin to die from exposure. Additionally, if toxic effects can be discerned before lethality occurs, remedial measures that avert mortality may still be possible. This work, therefore, focused on the chronic, sublethal effects of DDE and select PCB congeners in the midges, *Chironomus riparius,* in partial-life cycle tests. This approach has the additional benefit of providing body residues associated with sublethal effects of DDE and select PCB congeners in the midges, which can be used to create more nuanced assessment of hazard associated with the presence of the contaminants than is currently possible.

Comparison of chronic responses should be made in light of acute and chronic mortality data, where available, to assess the sensitivity of all endpoints. In the case of DBCP, the 35-day LR_{50} (0.68 mmol/kg) and the 10-day LR_{50} (1.6 mmol/kg) (Hwang 2000) span the range of the observed sublethal effect concentrations. While developmental effects were observed,

Table 5. Fecundity of adult female midges (average number of ova ± SD); Numbers with different superscripts are statistically different

Contaminant concentration on algae	DDE	DCBP	TCBP	PCBP
0	353.0 ± 70.7[a]	371.4 ± 55.5[a]	297.2 ± 50.7[a]	374.3 ± 57.7[a]
1	241.6 ± 36.3[b]	330.4 ± 58.7[a]	298.8 ± 34.9[a]	366.3 ± 64.4[a]
2	226.8 ± 60.5[b]	351.1 ± 52.7[a]	322.5 ± 68.7[a]	361.7 ± 65.1[a]
3	226.6 ± 39.4[b]	371.8 ± 65.8[a]	307.9 ± 40.9[a]	359.4 ± 51.2[a]
4	220.8 ± 37.6[b]	298.0	296.1 ± 63.7[a]	332.6 ± 77.9[a]
5	217.9 ± 45.2[b]	N/A	335.1 ± 40.6[a]	347.5 ± 65.6[a]

Contaminant concentrations on algae corresponding to each number are identified in Table 1.

the body residues needed to cause the effects were not different from those causing mortality. A similar logic can be applied to the TCBP data where the 95% CI for mortality at 10 days spanned the range of body residues needed to cause sublethal responses except for the female development to the adult (Figure 1). It was not possible to make this comparison for PCBP-exposed midges as the mortality data were not available. However, if one considers the lethal body residues of midges to TCBP (10-day LR_{50} 0.19 mmol/kg) and to HCBP (0.57 mmol/kg) (Hwang *et al.* 2001) and assumes that the 10-day LR_{50} for PCBP lies in between the lethal body residues for these two compounds, then it appears that sublethal effects will occur at lower body residues than are required to produce mortality in 10-day exposures. This is consistent with the observations of midges exposed to HCBP where the sublethal effects, particularly impaired fecundity, occurred at body residues that were lower than those required for 50% mortality at 10 days (Hwang *et al.* 2001). In the case of DDE (Figure 1), there is a clear difference in the body residues required to produce sublethal endpoints and that for mortality at 10 days. The fecundity endpoint was particularly sensitive compared to mortality; it required 100-fold more DDE to produce significant mortality than to significantly depress the production of ova.

Sublethal effects in invertebrates have occasionally been tracked. For instance, reductions in biomass and reproduction have been measured in *Lumbriculus variegatus* exposed to nonpolar narcotics (Nebeker *et al.* 1989). In addition, subtle changes in behavior in response to endrin exposure have been screened in *Limnodrilus hoffmeisteri* and *Stylodrilus heringianus* (Keilty *et al.* 1988) as well as *L. variegatus* exposed to pyrene (Kukkonen and Landrum 1994). Sublethal physiological impairments in *L. variegatus* have likewise been screened (Penttinen *et al.* 1996). However, in most cases, body residues at which these effects were realized were not measured.

Studies, that have connected body residues with sublethal effects in invertebrates, give contrasting results. For instance, Nebeker *et al.* (1989) found that numbers of *L. variegatus* with a body residue of 1.06 mmol/kg of hexachlorobenzene were reduced compared to untreated controls. However, the reduction in number was not statistically significant. Similarly, no significant sublethal effects were detected in *Chironomus tentans* or *L. variegatus* at body residues of TCDD as high as 9533 ng/g (West *et al.* 1997). In contrast, Fisher *et al.* (1999) found significant sublethal impairments of both growth and reproduction in *L. variegatus* exposed to five different PCBs at body residues that ranged from 0.34 to 0.56 mmol/kg in 35-day studies. These limited data suggest that significant impairments in sublethal parameters may be anticipated when body residues reach a critical level. Between-species variability in sublethal responses appears to be a fruitful area for future exploration.

Body Mass

Body mass was the least impacted test endpoint studied in both the second instar to pupae and the second instar to adult tests. No significant decreases in body weight with increasing body residues were found, although increases in body mass were found in female pupa exposed to TCBP and male adults exposed to DCBP, TCBP, and PCBP. These increases in body mass may be attributed to the high mortality at high exposure concentrations. The midges that metamorphosed to pupae or adults in the early stage of the experiments were found to be heavier than their counterparts in the later stage of the tests and more midges died in the later stage of the experiment as midges obtained lethal body residues. Consequently, the body mass at concentrations experiencing high mortality was greater than that at lower exposure concentrations. The lack of sensitivity of body mass to contaminant exposure may be attributable to the insect-specific physiology. The onset of metamorphosis to pupal stage in most insects is triggered by obtaining a critical body mass (Denlinger and Zdarek 1994). If the critical weight is not achieved, the metamorphosis does not occur. Therefore, the weights of midges upon metamorphosing to pupae and subsequently eclosing to adults should be similar regardless of treatment. Ristola and Kukkonen (1999) also reported that the weight of adult *C. riparius* did not change with increasing body residues of 2,4,5-trichlorophenol in chronic exposures.

The lack of sensitivity of body mass as an effective indicator of contaminant exposure seems to contradict the data of the USEPA (2000) which argued that weight depression should be a particularly useful measure of contaminant-induced effects. Weight depression is relatively simple to measure even in systems in which the route of exposure is through contaminated sediment from which the test organisms must be separated for biological assessment. Additionally, weight depression can serve as a surrogate for other sublethal parameters such as reduction in fecundity, which is more difficult to assess directly than weight loss. However, our data indicate that weight is not appreciably affected by contaminant exposure in our experiments.

There are several reasons why our data do not support the use of body mass depression as an endpoint for assessing

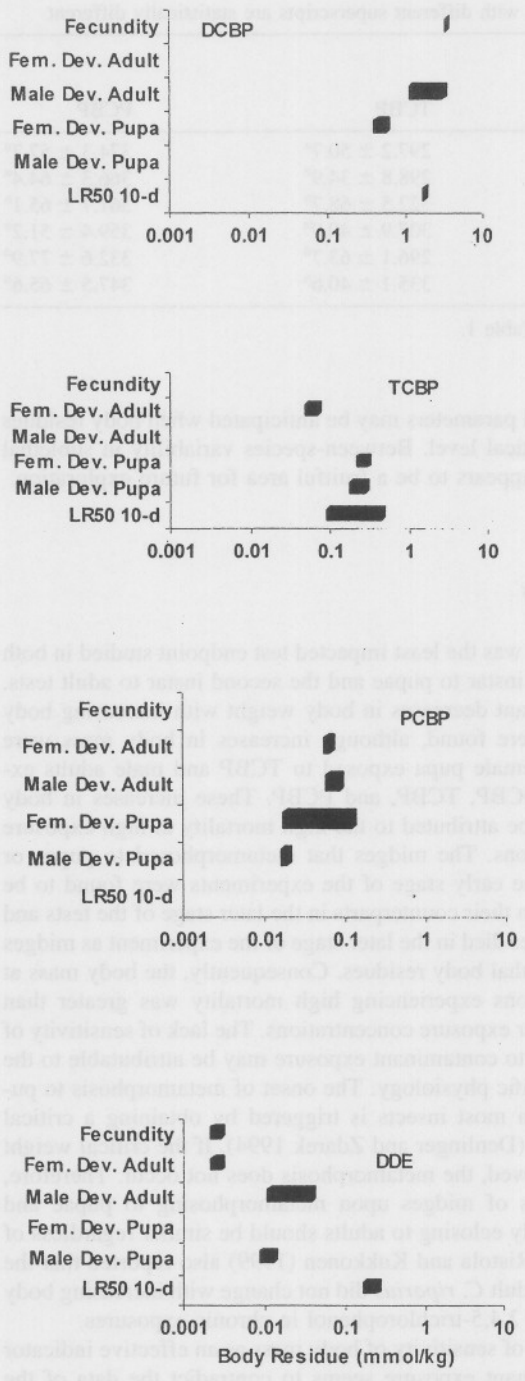

Fig. 1. The response spectrum for *Chironomus riparius* exposed to dichlorobiphenyl (DBCP), tetrachlorobiphenyl (TCBP), pentachlorobiphenyl (PCBP), and DDE, showing the 95% confidence interval range for each of the significant responses. Fecundity and the 10-day LR_{50} for DBCP were so variable that 95% confidence intervals could not be determined. The LR_{50} data were derived from a 10-day study on midges and are provided as a reference point for comparison with the sublethal endpoints from Hwang (2000).

sublethal effects of contaminant exposure in contrast to the conclusions of the USEPA (2000). First, there was a critical difference in our experimental designs. In the USEPA study (2000), organisms were exposed to different contaminant concentrations but organisms were assessed after a constant exposure time. In our work, midges were evaluated at the end of each stadium or when they metamorphosed to pupae or adults. Thus, the exposure duration was not constant among doses in that organisms at lower doses reached developmental endpoints more rapidly and were assessed earlier in the experiment than organisms that metamorphosed later. Thus, midges with slower rates of development (longer developmental times) would be smaller if the test were ended at a prescribed time that did not allow for metamorphosis to the next stage. Another difference between our study and that by the USEPA (2000) is that the USEPA (2000) employed organisms that did not undergo complete metamorphosis (*Lumbriculus terrestris* and *Hyalella azteca*). In complete metamorphosis, the body burden of contaminant and body mass may drop at each molt (Fisher *et al.* 1999). Thus, animals in the early part of a later instar may weigh less than they did at the end of the previous stage from which they have just metamorphosed. Body mass and contaminant load are lost in the exuvia that is cast off during the molt. Both body mass and contaminant load will be regained as the newly molted organism feeds and body mass expands to fill the new cuticle that is larger in size than was the case in the previous instar. Because of these variables, it is, thus, more difficult for body mass to mirror contaminant load or effects in organisms that go through complete metamorphosis.

Developmental Time

The increase in developmental time as a function of increasing body residues was the endpoint that showed the most consistent contaminant-induced impact among those evaluated. Development time was significantly increased in both the second instar to pupae and the second to adult tests (Figure 1). The increase in a developmental time may be due to energy allocation to stress from exposure to the contaminants. That is, the exposure to an environmental contaminant can be energetically costly to aquatic organisms. For instance, the metabolic rates of *C. riparius* increased upon exposure to narcotic chemicals such as 2,4-dichlorophenol and 2,4,5-trichlorophenol, suggesting some cost for exposure to the stressors (Penttinen *et al.* 1996; Penttinen and Kukkonen 1998).

Both TCBP and PCBP are coplanar congeners and were expected to be two of the more toxic PCB congeners tested. Coplanar congeners are known to have substantial 2,3,7,8-tetrachloro-*p*-dioxin (TCDD)-like activity and are significantly more toxic than noncoplanar congeners (Newsted *et al.* 1995; Safe 1994) due to their ability to bind cytosolic arylhydrocarbon (*Ah*) receptor (Bishop *et al.* 1998). However, West *et al.* (1997) did not find significant effects of TCDD on sublethal endpoints including organism weight and emergence time in *L. variegatus* at body residues of up to 9533 μg/kg lipid or 0.029 mmol/kg lipid and for *C. tentans* at 6876 μg/kg lipid or 0.023 mmol/kg lipid. The absence of effect was attributed to the absence of *Ah* receptor in aquatic invertebrates. That is, TCDD did not apparently exert its toxicity via receptor-mediated response, but by narcosis. The concentrations of TCDD obtained were an order of magnitude below that required to produce chronic narcosis (McCarty and Mackay 1993), thus explaining

the lack of effect. In contrast, developmental effects of the noncoplanar HCBP in midges occurred at concentrations similar to those of the coplanar compounds used in this study (Hwang *et al.* 2001). Thus, our data suggest that the coplanar and noncoplanar PCBs used in this study were acting as narcotics rather than through binding to the *Ah* receptor to increase in developmental time in the midge.

While there are very few data, the body residues required to produce sublethal responses in the midge compared to other invertebrate species are lower, making the midge more sensitive to this impact. The sublethal effects of DDE and select PCBs on growth in *L. variegatus* were assessed both by measuring the biomass of test organisms over time and by counting the number of organisms present (Fisher *et al.* 1999). *L. variegatus* reproduces by budding off, resulting in a doubling of the number of organisms every 20 days or so under normal conditions. In long-term tests, sublethal impairments will be manifest both in reductions of total organism numbers and in weight depression of individual organisms. For *L. variegatus,* significant reductions in organism number and biomass were found at body residues that varied between 0.34 and 0.56 mmol/kg for the five PCBs in a 35-day study. (Fisher *et al.* 1999). The body residues of *L. variegatus* that experienced significant growth reduction were higher, by a factor of 10, than those of *C. riparius,* which showed significant growth reduction. This difference is likely attributable to two factors. First, assessment of biomass in the adult midge yields a cumulative record of all the metamorphic events that preceded the adult stage. This is likely to produce a different snapshot than an assessment of an animal that does not undergo molting and for which growth is estimated from both biomass and organism number. However, it also must be acknowledged that *L. variegatus* appears to be less sensitive to the toxic effects of narcotic contaminants. This conclusion is supported by the failure to kill *L. variegatus* in 10-day toxicity tests with five PCBs (Fisher *et al.* 1999), while more than 85% *C. riparius* mortality was observed at the highest exposure concentrations for all the test chemicals except for DCBP in 10-day toxicity tests (Hwang 2000).

There have been some studies to determine the body residues related to sublethal effects of DDE on fish. For example, the survival and growth of the brook trout, *Salvelinus fontinalis,* were not affected by injected *p,p'*-DDE at body residues between 0.003 and 0.016 mmol/kg over 28 days (Addison *et al.* 1977). The body residue range of DDE in the work of Addison *et al.* (1977), 0.003–0.016 mmol/kg, includes an LOER for female developmental time and fecundity changes. Thus, the midge seems to be more sensitive than fish to these compounds particularly for sublethal endpoints, as significant responses in development time occur at lower body residues.

These developmental time increases due to chemical stress may cause a significant reduction in the midge population in the environment due to increased chance of predation a from prolonged period of presexual maturation. The reduction in growth coupled with reduction in fecundity can cause population reduction, which may increase the chance of extirpation (Klok and Roos 1996). For instance, Hallam (1993) showed that sublethal stress imposed by narcotic chemicals to the individual organisms could cause population extinction in an experiment using a model daphnia population.

Fecundity

Reductions in fecundity, expressed by the number of ova in each female midge, occurred in DDE-exposed midges, while no significant fecundity reductions were detected in the midges exposed to DCBP, TCBP, and PCBP (Table 5). In addition, LOERs of DDE for female developmental time and fecundity changes were lower than other sublethal endpoints by more than a factor of 10, while the LOER of DDE for male developmental time was very close to the LOERs determined for PCBs. The effect of DDE on fecundity reduction may be attributable to endocrine disruption (Kelce *et al.* 1995). In fact, *p,p'*-DDE is known to be a strong anti-androgen receptor antagonist (Kelce *et al.* 1995; Sharpe 1995), although it is not strongly antiestrogenic (Donohoe and Curtis 1996). However, the functions of hormones in insects are very different from those in mammals. That is, the functions estrogen and androgen are not known in insects, even though their presence in insects has been confirmed (Blum 1985). Therefore, *p,p'*-DDE may not cause endocrine disruption in insects in the same manner as it does in mammals or fish. PCBs have also been reported to possess endocrine disrupting activities in reptiles (Crain and Guillette 1998), fish (Gerstenberger *et al.* 2000), mammals (Morse *et al.* 1996), and humans (Krishnam and Safe 1993). In addition, several studies have addressed the effects of PCBs on fecundity in invertebrates (DePinto *et al.* 1993; Fisher *et al.* 1999; Hwang 2001). However, no effects of PCBs on fecundity were found in the present work (Table 5) despite the fact that the TCBP and PCBP were coplanar congeners that were expected to be more biologically active. These data are also contrary to the finding that HCBP had significant effects on fecundity at 0.02 mmol/kg in both pupal and adult midges (Hwang *et al.* 2001). In addition, Fisher *et al.* (1999) found that fecundity was significantly reduced in *Lumbriculus variegatus* exposed to MCBP, TCBP HCBP, and DCBP at body residues of 0.34–0.52 mmol/kg. Thus, it could be that the body residues achieved in this study were simply too low to exert endocrine disrupting activity, which subsequently reduces fecundity.

Conclusions

Of the chemicals tested, DDE was the most biologically active contaminant, producing significant reductions in fecundity and significant delays in developmental time for male and female midges. Further, these effects were realized at comparatively low body residues (0.003 mmol/kg and greater) In contrast, DCBP was the least toxic contaminant for which the only evidence of toxicity was a significant delay in development time at body residues of 0.27 mmol/kg or greater. Body mass of midges was not significantly impacted by exposure to any contaminant used in this study. The expression of chronic sublethal effects at body residues significantly lower than the literature values for acute mortality for DDE and the higher chlorinated PCB congeners suggests that the ecological integrity can be affected at levels below those that produce mortality. The increased developmental time and decreased fecundity may affect the midge population negatively by increasing chance of predation before reproduction. These ecologically important parameters should, therefore, be assessed in ecological risk assessment studies.

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